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Cross-resistance between cyenopyrafen and pyridaben in the twospotted spider mite *Tetranychus urticae* (Acari: Tetranychidae)

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Running title: Cross-resistance between cyenopyrafen and pyridaben in *T. urticae*

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Abstract

BACKGROUND: Cyenopyrafen is an inhibitor of complex II of the mitochondrial electron transport chain. It has a molecular structure that shares some common features with frequently used complex I inhibitors such as pyridaben. To evaluate whether this similarity in structure poses a cross-resistance risk that might complicate resistance management, we selected for pyridaben and cyenopyrafen resistance in the laboratory and characterized resistance.

RESULTS: The selection for cyenopyrafen conferred cross-resistance to pyridaben and vice versa. Resistance towards these both acaricides was incompletely dominant in adult females. However, in eggs maternal effects were observed in pyridaben resistance, but not in the cyenopyrafen-resistance (completely dominant). In the cyenopyrafen resistant strain, the LC_{50} of eggs remained lower than the commercially recommended concentration. The common detoxification mechanisms by cytochrome P450 was involved in resistance to these acaricides. Carboxyl esterases were also involved in cyenopyrafen resistance as a major factor.

CONCLUSIONS: Although cross-resistance suggests that pyridaben resistance would confer cyenopyrafen cross-resistance, susceptibility in eggs functions to delay the development of cyenopyrafen resistance.

Keywords: acaricide resistance; cross-resistance; cyenopyrafen; pyridaben; *Tetranychus urticae*

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1 INTRODUCTION

The twospotted spider mite *Tetranychus urticae* Koch is an economically important pest in many agricultural crops, since it rapidly develops resistance to newly developed acaricides. Spider mite control and resistance management has become complicated due to cross-resistance that is often observed among acaricides with similar mode of action and by the presence of strains resisting most distinctive acaricidal classes (multi-resistance).[1,2]

Cyenopyrafen is a mitochondrial complex II electron transport inhibitor that was commercialized in 2009.[3–6] To the best of our knowledge, cyenopyrafen resistance in *T. urticae* has not been reported. On the other hand, mitochondrial complex I electron transport inhibitors (complex I inhibitors) including pyridaben, tebufenpyrad, and fenpyroximate were commercialized in the early 1990s and have ever since been frequently used worldwide. Although the target sites are distinctive, cyenopyrafen is composed of a molecular structure common to complex I inhibitors: one pyrazole ring and one tertiary butyl group.

Cross-resistance among complex I inhibitors had been reported in several previous studies.[7–10] Stumpf and Nauen[10] pointed out that common molecular structures among the complex I inhibitors, specifically heterocyclic rings with two nitrogen atoms associated with long hydrophobic tail structures with at least one tertiary butyl group, are a possible cross-resistance factor. The synergism of piperonyl butoxide (PBO) on toxicity, together with the documentation of increased cytochrome P450 activity, suggest that metabolism by cytochrome P450 is one of the major (cross-)resistance mechanism to complex I inhibitors in *T. urticae*.[7] Therefore, the question whether the similarity in structure between cyenopyrafen and the complex I inhibitors would also result in cross-resistance

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is the objective of this study.

We tested whether cross-resistance would occur between cyenopyrafen and pyridaben. First, we selected a field collected *T. urticae* population with both acaricides separately, and tested whether selection by cyenopyrafen causes loss of susceptibility to pyridaben or vice versa. Then, we investigated the mode of inheritance of resistance, and tested the synergetic effects of detoxification enzyme inhibitors. From these results, we discuss the mechanisms of cross-resistance and the associated risks in mite management.

2 MATERIALS AND METHODS

2.1 Chemicals

The acaricides used in this study were commercial formulations of cyenopyrafen (Starmite,® 30 SC) and pyridaben (Sanmite, ® 20 SC). Chemicals were suspended in appropriate volumes of distilled water.

Synergists used to evaluate the role of detoxification enzymes were PBO (90%; a cytochrome P450s inhibitor), *S*-benzyl-*O*,*O*-diisopropyl phosphorothioate (IBP, 98%; a carboxyl esterase inhibitor), triphenyl phosphate (TPP, 97%; a carboxyl esterase inhibitor), and diethylmaleate (DEM, 97%; glutathione *S*-transferase inhibitor). All these synergists were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

2.2 Mites

A field population (NO) of *T. urticae* was originally collected from roses in a greenhouse in Heguri, Nara Prefecture, Japan (34°37′N, 135°42′E), in May 2010. The mites on the roses had been sprayed mainly with dienochlor and occasionally with etoxazole, hexythiazox, chlorfenapyr, acequinocyl, bifenazate, emamectin benzoate, or milbemectin.

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On the other hand, cyenopyrafen, pyridaben, and cyflumetofen had never been used before May 2010.

An acaricide susceptible strain (NS) had been originally collected from chrysanthemum (*Chrysanthemum morifolium* R.) in Katsuragi, Nara Prefecture, Japan (34°30′N, 135°43′E) in 1998. NS was established as a susceptible strain after adversely selecting for increased susceptibility to both etoxazole and hexythiazox in a laboratory by Asahara et al.[11] and then reared under acaricide-free conditions until this study.

All strains and stock cultures were reared on detached kidney bean (*Phaseolus vulgaris* L.) leaves placed on water-soaked cotton in Petri dishes (9 cm diameter), in the laboratory at 25°C, 60 % relative humidity, and 16:8 h light and dark photoperiod.

2.3 Laboratory selections and cross-resistance

Laboratory selection with cyenopyrafen and pyridaben was performed separately to obtain resistant strains (R) to each acaricide and to evaluate the effects of selection by one acaricide on the susceptibility to the other acaricide (cross-resistance). Prior to selection, we prepared two subpopulations derived from the NO culture. Then, one subpopulation was selected with cyenopyrafen six times, and the other was exposed to pyridaben five times. The concentration of acaricides applied to each selection was gradually increased with progression of the selection, i.e., in the order of 75, 150, 1000, 1500, 1500, and 1500 mg/L for cyenopyrafen, and 200, 1000, 10000, 10000, and 10000 mg/L for pyridaben.

Five fresh kidney bean leaf discs, each containing more than 200 mites of various developmental stages, were separately dipped into acaricide solution for 10 s, dried on a paper towel at room temperature, and then replaced on water-soaked cotton in Petri

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dishes. Five days later, adult females that survived on acaricide-treated leaf discs were moved to newly prepared kidney bean leaf discs with a fine brush and the population was allowed to increase. The subsequent selections were performed at 14–day intervals. The strains obtained after the selection with cyenopyrafen (NCR) and pyridaben (NPR) were separately reared on kidney bean leaf discs (~5 cm in diameter) without additional selections.

2.4 Toxicological tests

2.4.1 Ovicidal bioassay

Ten adult females were introduced to a kidney bean leaf disc prepared as described above and were allowed to oviposit under laboratory conditions. After 24 h the females were removed from the leaf disc. Then, the leaf disc with eggs was dipped into acaricide solution for 10 s. After being dried on a paper towel at room temperature, the leaf disc was replaced on water-soaked cotton in the Petri dish. Mortality was calculated 7 days after acaricide treatment by counting the number of unhatched eggs.

Approximately 60–100 eggs were present per leaf disc. Three leaf discs were used per concentration for each strain and acaricide. The data of the three leaf discs were pooled and analyzed as a no replication experiment. Mortality rates were corrected using Abott's formula.[12] The results were analyzed by probit regressions to determine the 50% lethal concentration (LC_{50}) values and 95% fiducial limits that were calculated using a program for the 50% effective dose (ED_{50}; http://aoki2.si.gunma-u.ac.jp/R/ed50.html) by Aoki[13] with some modifications using R software.[14] Resistance factors (RFs) were calculated by dividing the LC_{50} value for each selected strain (NCR or NPR) by the LC_{50} value of a susceptible strain (NS).

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96

97 *2.4.2 Adultcidal bioassays*

98 Ten adult females were moved from mite culture to a kidney bean leaf disc (2 × 2 cm)
99 and allowed to settle for 30 min. The leaf disc with adult females was dipped into
100 acaricide solution for 10 s, dried on a paper towel at room temperature, and then replaced
101 on water-soaked cotton in a Petri dish. Distilled water without acaricide was used as
102 control. The number of survivors was counted under a binocular microscope 5 days after
103 the acaricide treatment. Mites that could move normally were scored as alive while mites
104 that were paralyzed after touching with a fine brush were scored as dead. Individuals that
105 escaped from leaf discs were excluded from data analyses.

106 Six leaf discs were used per concentration for each strain and acaricide. The data of
107 the six leaf discs were pooled and analyzed as a no replication experiment. These results
108 were analyzed in the same way as described for the ovicidal bioassay.

109

110 **2.5 Crosses to determine the mode of inheritance**

111 To test dominance and maternal effects of resistance, the resistant strain (NCR or NPR)
112 was reciprocally crossed with the susceptible strain (NS). Then, a toxicological test was
113 applied to eggs and females of the parental strains and F_1 generations derived from the
114 reciprocal crosses.

115 Sixty teleiochrysalid females of one strain and 60 adult males of the other strain
116 were randomly chosen from each culture and introduced to a fresh kidney bean leaf disc
117 using a fine brush. Females were usually inseminated immediately following their last
118 molt. After 3 days, to obtain F_1 eggs, the crossed females were transferred onto a new
119 leaf disc and allowed to oviposit for 24 h under laboratory conditions. Cyenopyrafen and

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pyridaben susceptibility of the F_1 eggs was evaluated by the ovicidal bioassay.

To obtain hybrid F_1 females, the crossed females described above were moved to a new leaf disc. After 24 h, the parental females were removed, and F_1 eggs laid on the leaf disc were reared to adulthood. Cyenopyrafen and pyridaben susceptibility of the F_1 adult females was evaluated by the adulticidal bioassay.

The degree of dominance (D) was calculated using a formula of Stone (1968):[15]

$$D = \frac{2Y - X - Z}{X - Z},$$

where X is the logarithmic LC_{50} value of the resistant strain, and Y and Z are the LC_{50} values of F_1 females and the susceptible strain, respectively. The D values should range from −1 (resistance inherits completely recessive) to 1 (completely dominant).[15] Because of arrhenotokous parthenogenesis in *T. urticae*, F_1 eggs produced from R♀ × S♂ and S♀ × R♂ crosses should contain resistant and susceptible male eggs, respectively. Therefore, the LC_{50} values were not determined for the F_1 eggs.

2.6. Synergism tests

Synergists were dissolved in aqueous acetone (1:1) and sprayed on a leaf disc (2 cm in diameter) containing 10 adult females using a glass chromatograph sprayer (0.3 mL per leaf disc). After 4 h of synergist treatment, the females were applied to the adulticidal bioassay, and their LC_{50} value was determined. To minimize the effects of the synergist itself, the concentrations of synergists used for the treatments were settled lower than the LC_{10} of NS at 250, 100, 250, and 500 mg/L for PBO, IBP, TPP, and DEM, respectively, based on preliminary experiments.

The synergistic ratio (SR) was calculated by dividing the LC_{50} value without the

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synergist by the LC_{50} value with the synergist. If the 95% confidence limits of the LC_{50} values did not overlap between without and with the synergist, then the synergistic effect was considered to be significant.

3. Results

3.1. Laboratory selections and cross resistance

LC_{50} values of NS were below 1 and 4 mg L^{-1} in eggs and adult females, respectively, for both cyenopyrafen and pyridaben (Table 1). A moderate degradation of cyenopyrafen susceptibility had been occurred in NO (LC_{50} values and RFs were 59.34 mg L^{-1} and 24.52, respectively, in adult females and 35 mg L^{-1} and 140, respectively, in eggs). In contrast, no decrease in LC_{50} toward pyridaben was found in NO. However, the slopes of the pyridaben concentration–mortality regression lines in adult females were smaller in NO than NS (Table 1). Moreover, the mortality from 10000 mg L^{-1} pyridaben in adult females of NO calculated from the concentration–mortality regression line was 74.1%, indicating the heterogeneity of NO in pyridaben resistance.

Senior author (MO) with a colleague tentatively studied acaricide susceptibility of *T. urticae* population collected from the same greenhouse in June 2009 (only four months after commercialization of cyenopyrafen in Japan). They found survivability more than 80% in adult females after application of cyenopyrafen to adult females at the concentration of 150 mg L^{-1}, although all eggs died (Uesugi and Osakabe unpublished data). Moreover, serious or moderate degradation of efficacy was also found in cyflumetofen, bifenazate, acequinocyl, milbemectin, and tetradifon in 2009 (Uesugi and Osakabe unpublished data), suggesting the potential development of multiple resistances in NO. The moderate degradation of cyenopyrafen susceptibility and the heterogeneity of

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pyridaben susceptibility in NO were also potentially caused by the multiple resistances.

After laboratory selection with cyenopyrafen (NCR), the LC_{50} for cyenopyrafen reached 103.68 and 1502.82 mg L^{-1} (RF = 414.72 and 621; 3- and 25-fold of NO) in eggs and adult females, respectively (Table 1). LC_{50} of NCR for pyridaben also increased to 1454.98 and >10000 mg L^{-1} (RF = 1914.45 and >2583.98) in eggs and adult females, respectively. However, the slope of the pyridaben concentration–mortality regression lines for NCR (0.40 in eggs and 0.24 in adult females) were smaller than that of NS (Table 1). Moreover, the mortality of 10000 mg L^{-1} pyridaben calculated from the concentration mortality regression line was 34.6% and 63.1% for the adult females and eggs of NCR, respectively. This result indicates the locus (or loci) involved with pyridaben resistance might remain heterogeneous in NCR.

For NPR, the LC_{50} of both eggs and adult females exceeded 10000 mg L^{-1} for pyridaben; mortality was 3.4% at 10000 mg L^{-1} (n = 59, corrected mortality = 0%; mortality of control = 3.4%, n = 58). Therefore, calculating LC_{50} and obtaining a formula for concentration–mortality regression lines were impossible. The LC_{50} values of NPR eggs and adult females for cyenopyrafen increased to 74.16 and 430.99 mg L^{-1} (RF = 296.64 and 178.10), respectively.

3.2 Mode of inheritance

3.2.1 Eggs

For cyenopyrafen, the mortality–concentration regression lines of F_1 eggs produced by NCR♀ × NS♂ were close to that of NCR (Fig. 1a). In F_1 eggs from NS♀ × NCR♂, a part of the eggs showed a mortality rate similar to that of NS, whereas the remaining eggs showed mortality similar to NCR. This division was rational because of

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arrhenotokous parthenogenesis in this mite; haploid male eggs produced by NS♀ should be cyenopyrafen-susceptible. Therefore, cyenopyrafen resistance in the eggs was determined to be completely dominant.

We could not represent the plots of mortality for pyridaben or the mortality–concentration regression line for NPR because LC_{50} was too high. Mortality of F_1 eggs from NS♀ × NPR♂ plotted near the mortality–concentration regression line for NS (Fig. 1b). In contrast, F_1 eggs produced by NPR♀ × NS♂ showed obviously higher tolerance.

To confirm the reproductive compatibility between NPR and NS, we additionally performed intra- and inter-strain crosses. We placed 60 teleiochrysalid females and 30 adult males together on a leaf disk for three days. Then, 20 adult females (randomly chosen from the emerged adult females) were allowed to oviposit for one day. Oviposited eggs were reared until adulthood, and sex ratios were checked under a binocular microscope. As a result, we obtained similar sex ratios from all reciprocal crosses (NPR♀ × NPR♂: 241 eggs, development = 93.8%, sex ratio (females/total) = 0.74; NPR♀ × NS♂: 248, 93.5%, 0.75; NS♀ × NPR♂: 191, 93.2%, 0.72; NS♀ × NS♂: 175, 89.1%, 0.75), indicating that no reproductive incompatibility was involved in the results of crosses between these strains. Therefore, we consider that some maternal factors play a role in pyridaben resistance.

3.2.2 Adult females

The mortality–concentration regression lines of cyenopyrafen for F_1 females from both NCR♀ × NS♂ and NS♀ × NCR♂ appeared closely to NCR (Fig. 2a). The LC_{50} values corresponded to each other between the reciprocal crosses, and the degree of dominance

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of resistance (*D*) was 0.47 and 0.50 in F_1 females from NCR♀ × NS♂ and NS♀ × NCR♂, respectively (Table 2). Therefore, the inheritance of cyenopyrafen resistance in adult females was estimated to be incompletely dominant.

For pyridaben, the LC_{50} values of F_1 females from the reciprocal crosses were obviously higher than those of NS (Table 2, Fig. 2b), suggesting that pyridaben resistance was incompletely dominant.

3.3 Synergism test

Pretreatment of PBO and TPP resulted in high synergistic effects on cyenopyrafen toxicity in the NCR strain. The LC_{50} of NCR for cyenopyrafen (1502.82 mg L^{-1}) was reduced to 18.74 and 22.01 mg L^{-1} by PBO and TPP (SR = 80.19 and 68.28), respectively (Table 3). Lesser but significant synergistic effects were exhibited with IBP and DEM, and LC_{50} values were reduced to 734.15 and 551.25 mg L^{-1} (SR = 2.05 and 2.73), respectively. This suggests that cyenopyrafen resistance in NCR is mainly linked with enhanced metabolism by cytochrome P450s and carboxyl esterases. Other carboxyl esterases inhibited by IBP and glutathione *S*-transferases are also potentially involved with the cyenopyrafen resistance of NCR as minor factors.

In the NPR strain, a clear synergistic effect was shown only when pretreated with PBO. The LC_{50} of NPR for pyridaben (>10000 mg L^{-1}) was reduced to 73.24 mg L^{-1} (SR > 136.54). No synergistic effects were observed from TPP, IBP and DEM treatments. Therefore, one of the main mechanisms of pyridaben resistance in NPR is detoxification by cytochrome P450s.

4 Discussion

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The LC_{50} value of cyenopyrafen was rapidly increased by a limited number of laboratory selections. The RFs increased to 25-fold in adult females and 3-fold in eggs, respectively, in comparison with the field collected parental population (NO). The mode of inheritance is incompletely (adult females) or completely (eggs) dominant, which potentially accelerate resistance development in general.[16] Reciprocal crossing revealed no maternal inheritance of cyenopyrafen resistance, indicating no involvement of genetic modification in the mitochondrial DNA. Also, there are no subunits of complex II encoded by the mitochondrial DNA.

Cyenopyrafen is pro-acaricide activated after hydrolysis by esterases[5] similar to cyflumetofen, another complex II inhibitor [17], and also bifenazate, a complex III inhibitor.[18,19] Indeed, slight increase of LC_{50} values was observed in NS treated with IBP and TPP. However, the effects of the esterase inhibitors were very small in comparison with the case of bifenazate when esterases were inhibited with another chemical, *S,S,S*-tributyl-phosphorotrithioate (DEF).[18] Esterases which activate cyenopyrafen might be less sensitive to IBP and TPP, as it has been shown that the level of esterase inhibition defers between inhibitors in *T. urticae*.[19] In contrast, pretreatment by TPP decreased LC_{50} of NCR to the concentration lower than the LC_{50} before laboratory selection (NO) as well as that by PBO. Pretreatment by IBP also halved the LC_{50} of NCR toward cyenopyrafen. Therefore, both cytochrome P450 and carboxyl esterases are essential for the detoxification of cyenopyrafen. On the other hand, a significant synergistic effect was obtained by PBO pretreatment, but the pretreatments with TPP, IBP, and DEM did not exert any influence toward pyridaben resistance levels in NPR. Synergism by PBO was commonly observed among the studies associated with the complex I inhibitors.[7,8,10,20] Our study suggests that the common molecular structures among the complex I inhibitors

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263 are also a possible cross-resistance factor between pyridaben (or other complex I
264 inhibitors) and cyenopyrafen, but unique mechanisms by carboxyl esterases are also
265 involved with cyenopyrafen resistance.

266 These results suggest that an application history of pyridaben or other complex I
267 inhibitors could potentially confer cyenopyrafen cross-resistance. However, although the
268 LC_{50} values of adult females were significantly higher than the commercially
269 recommended concentration of cyenopyrafen (150 mg L^{-1}) in both NCR and NPR, the
270 LC_{50} values of those eggs toward cyenopyrafen still remained lower than the
271 commercially recommended concentration. Therefore, application with cyenopyrafen at
272 the commercially recommended concentration can be expected to cause significant
273 mortality of eggs even after achieving some resistance levels in adult females.

274 A similar age-dependent expression of resistance (lower resistance levels in eggs)
275 has been recently reported in the resistance of *T. urticae*[21] and the European red mite
276 *Panonychus ulmi* Koch[22] against spirodiclofen, which is an acaricide that interfere with
277 lipid biosynthesis (expected acetyl-CoA carboxylase inhibitor).[23] Cytochrome P450 and
278 carboxyl esterase in *T. urticae* and only cytochrome P450 in *P. ulmi* were involved in the
279 detoxification process of spirodiclofen, respectively.[21,22] Demaeght et al.[24] revealed that
280 the expression levels of *CYP392E10*, that metabolizes spirodiclofen, were very low in
281 eggs compared to other life stages in *T. urticae*. Therefore, it would be interesting to
282 investigate whether the expression levels of the cyenopyrafen resistance related
283 cytochrome P450 gene are also low in eggs of the NCR strain.

284 In this study, we transferred adult females survived the selection with acaricides to
285 new leaf discs and allowed the mites to increase without additional chemical application,
286 resulting quick development of cyenopyrafen resistance in NCR. However, the

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susceptibility in eggs to cyenopyrafen is most likely to cause more effective decrease in the population sizes than the effects expected from the resistance levels of adult females. In a theoretical study, a higher degree of reduction delays the population increase and thus delays resistance development.[16] This might be true in *T. urticae* populations which have acquired resistance to pyridaben or other complex I inhibitors. Moreover, we found that carboxyl esterase inhibited by TPP were also essential for cyenopyrafen resistance, and that inhibited by IBP and glutathione *S*-transferase might partially contribute to expression of the resistance. Such resistance mechanisms were not likely to be selected by the application with pyridaben. Although significance of carboxyl esterase inhibited by DEF in pyridaben resistance had been reported by Van Pottelberge et al.[7], pretreatments with TPP and IBP had no effects on pyridaben resistance expression in NPR. Valles et al.[25] pointed out that DEF potentially inhibited not only esterases but also microsomal oxidases in German cockroach *Blattella gennanica* (L.), although this was never reported for mites. This might be a potential reason that, although complex I inhibitors had been widely used for the mite control, development of serious resistance against cyenopyrafen has never been reported in field *T. urticae* population in Japan for ≈4 years after the commercialization.

Another point of our findings is the significant maternal effects in the resistance levels toward pyridaben in eggs derived from the reciprocal crosses between NPR and NS. Complete maternal inheritance of acaricide resistance has been reported in the bifenazate-resistant Belgian population.[18] The maternal effects are caused by mutations in the mitochondrial cytochrome *b*, and the mutations confer cross-resistance toward acequinocyl.[26] However, although the maternal effect was supported in adult females in bifenazate resistance, the maternal effects in pyridaben resistance appeared in eggs but

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311 disappeared in adult females. Moreover, synergistic tests indicate that the detoxification
312 by cytochrome P450 is the major mechanism conferring pyridaben resistance. Therefore,
313 the mechanisms of such the age-dependent maternal effects remain still unclear.
314 Partial maternal effects on resistance were reported in the complex I inhibitors
315 (pyridaben and fenpyroximate) by Stumpf and Nauen.[10] However, the maternal effect
316 was not clearly supported and was not documented in subsequent studies, where maternal
317 inheritance was mainly evaluated in F_1 females.[7,9,27] Because ND1 and ND5 genes of
318 mitochondrial complex I subunits are encoded on mitochondrial DNA, if target-site
319 resistance would be in place most likely only ND1 and/or ND5 subunits are involved.
320 However, given that the maternal effects in eggs can be explained by such target-site
321 resistance, there are no reasons that such target site insensitivity cannot function as an
322 alternative resistance mechanism when the metabolism was inhibited by chemicals.
323 Additionally, no evidence has been reported in the complex I inhibitor resistance-related
324 mutation of ND1 and ND5.[2] It is worth investigating, if expression of the cytochrome
325 P450 gene involved in pyridaben resistance is low in eggs like as *CYP392E10*, what
326 factors can be conferring pyridaben resistance in eggs. Further studies including analyses
327 of target-site genetic modification in mitochondrial DNA and detoxification enzyme
328 activities in eggs will be required to elucidate mechanisms of the age-dependent maternal
329 effects in pyridaben resistance.
330
331 **Acknowledgements**
332 The authors thank Y. Kunimoto, Nara Plant Protection Center, and H. Fujieda for their
333 help to sample field population of *T. urticae*. We also thank T. Van Leeuwen who kindly
334 read the previous version of the manuscript and gave us a lot of valuable comments and

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suggestions.

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Figure legends

Figure 1. Concentration–mortality lines for cyenopyrafen (a) and pyridaben (b) in eggs of susceptible (NS) and resistant (NCR, NPR) strains and in F_1 eggs from reciprocal crosses between the susceptible and resistant strains, respectively; open and solid triangles represent NCR and NS strains, respectively. Open and solid circles represent F_1 eggs from R (♀) × S (♂) and S × R crosses, respectively. Data from NPR are not shown because its LC_{50} was too high to be determined (>10000 mg L^{-1}; see Table 1).

Figure 2. Concentration-mortality lines for cyenopyrafen (a) and pyridaben (b) in adult females of NS and resistant (NCR, NPR) strains and in F_1 adult females from reciprocal crosses between the susceptible and resistant strains. Open and solid triangles represent NCR and NS strains, respectively; open and solid circles represent F_1 adult females from R (♀) × S (♂) and S × R crosses, respectively. Data from NPR are not shown because its LC_{50} was too high to be determined (>10000 mg L^{-1}; see Table 1).

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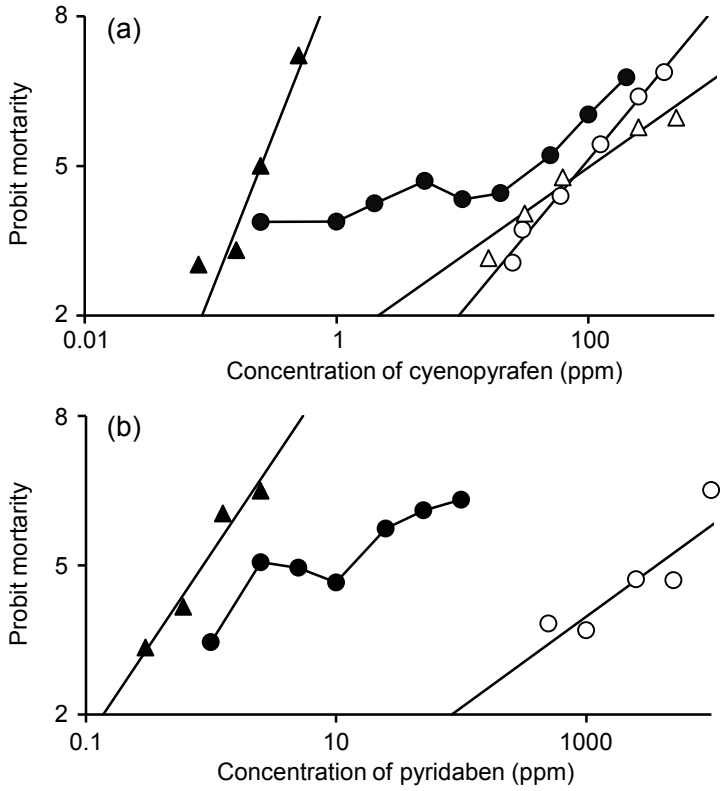

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439 Fig. 1
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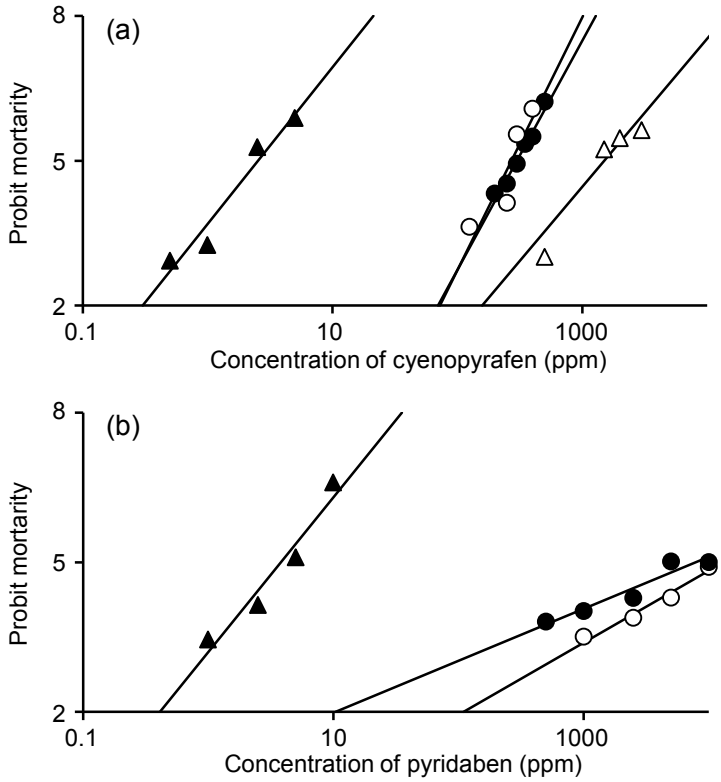

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444 Fig.2
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446 **Table 1** Logarithmic dose-probit mortality regression line data against cyenopyrafen
447 (Cye) and pyridaben (Pyr) expressed as LC_{50}, slope, and resistance factor (RF) in
448 acaricide-susceptible strain (NS), field collected population (NO), and strains selected by
449 pyridaben (NPR) and cyenopyrafen (NCR)

Strains	Acaricides	Developmental stages tested	LC_{50} values (mg/L)	95% fiducial limits of LC_{50} values	Regression lines	RF
NS	Cye	Egg	0.25	0.24–0.26	Y = 6.40 X + 8.84	1
		Adult female	2.42	2.08–2.83	Y = 3.38 X + 3.70	1
	Pyr	Egg	0.76	0.717–0.80	Y = 3.8 X + 5.46	1
		Adult female	3.87	3.29–4.57	Y = 3.07 X + 3.19	1
NO	Cye	Egg	35.00	29.27–43.19	Y = 1.31 X + 2.98	140
		Adult female	59.34	51.60–69.58	Y = 2.60 X + 0.40	24.52
	Pyr	Egg	0.42	0.38–0.46	Y = 1.90 X + 5.71	0.55
		Adult female	2.24	0.00–37.64	Y = 0.21 X + 4.93	0.58
NCR	Cye	Egg	103.68	94.01–114.47	Y = 1.78 X − 4.75	414.72
		Adult female	1502.82	1323.69–1707.14	Y = 3.07 X + 1.41	621
	Pyr	Egg	1454.98	947.72–2222.77	Y = 0.40 X + 3.74	1914.45
		Adult female	>10000	—	Y = 0.24 X + 3.66	>2583.98
NPR	Cye	Egg	74.16	68.59–80.02	Y = 2.86 X − 0.36	296.64
		Adult female	430.99	347.22–547.35	Y = 1.91 X − 0.03	178.10
	Pyr	Egg	>10000	—	—	>13157.89
		Adult female	>10000	—	—	>2583.98

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452 **Table 2** Logarithmic dose-probit mortality regression line data against cyenopyrafen
453 (Cye) and pyridaben (Pyr) expressed as LC_{50}, slope, and degree of dominance of
454 resistance (*D*) in F_1 adult females produced by reciprocal crosses between NS and NCR,
455 and between NS and NPR strains

Acaricides	Crosses (♀ × ♂)	LC_{50} values for F_1 females (mg/L)	95% fiducial limits of LC_{50} values	Regression lines	*D*
Cye	NCR × NS	271.28	249.02–294.60	Y = 5.24 X − 7.74	0.47
	NS × NCR	299.47	277.37–321.38	Y = 4.76 X − 6.78	0.50
Pyr	NPR × NS	>10000	8610.80–>10000	Y = 1.45 X − 0.95	—
	NS × NPR	7848.20	4972.79–>10000	Y = 1.04 X + 0.93	—

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457 **Table 3** Synergistic effects of PBO, IBP, TPP, and DEM on adult females of NS, NCR,
458 and NPR treated with cyenopyrafen (Cye) and pyridaben (Pyr)

Strains	Acaricides + Synergists	LC_{50} values (mg/L)	95% fiducial limits of LC_{50} values	Regression lines	Synergistic ratios
NS	Cye	2.42	2.085–2.83	Y = 3.38 X + 3.70	1
	+ PBO	2.08	1.49–3.27	Y = 1.14 X + 4.64	1.16
	+ IBP	12.12	10.20–14.65	Y = 2.58 X + 2.21	0.20
	+ TPP	21.70	19.0–25.1	Y = 3.20 X + 0.64	0.11
	+ DEM	1.39	1.17–1.64	Y = 3.09 X + 4.56	1.74
NCR	Cye	1502.82	1323.69–1707.14	Y = 3.07 X − 4.75	1
	+ PBO	18.74	15.46 – 22.48	Y = 2.25 X +2.14	80.19
	+ IBP	734.15	602.09–869.34	Y = 2.16 X − 1.18	2.05
	+ TPP	22.01	16.61–28.21	Y = 1.43 X + 3.07	68.28
	+ DEM	551.25	464.71–662.90	Y = 2.41 X − 1.16	2.73
NS	Pyr	3.87	3.29–4.57	Y = 3.07 X + 3.19	1
	+ PBO	0.09	0.07–0.12	Y = 1.49 X + 6.56	43
	+ IBP	2.79	2.05–4.07	Y = 1.65 X + 4.27	1.39
	+ TPP	1.36	0.97–2.00	Y = 1.28 X + 4.83	2.85
	+ DEM	3.04	2.33–4.50	Y = 2.08 X + 4.00	1.27
NPR	Pyr	>10000	—	—	1
	+ PBO	73.24	53.31–109.73	Y = 1.37 X + 2.44	>136.54
	+ IBP	>10000	—	—	1.00
	+ TPP	>10000	—	—	1.00
	+ DEM	>10000	—	—	1.00

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